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Experimental Studies of Essential Hemospermia with Special Reference to the Fibrinolytic Activity

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Abstract

The fibrinolytic activity of the allergic seminal vesiculitis of rabbits induced by the complete adjuvant method using egg albumin was studied by the topographical and the fibrin plate method. A remarkable increase of plasminogen activator was observed in the mucous layer having the allergic reactions. A similar result was also obtained by the estimation of the tissue activator activity. Consequently, the plasminogen activator of the seminal vesicle secretion significantly increased. In addition, the determination of the streptokinase-activated fibrinolytic activity revealed a remarkable increase of proactivator in both the vesicle tissue and secretion. There was no increase of fibrinolytic activity in the blood. The inhibitory effect of trans-AMCHA upon these enhanced fibrinolytic activities was sufficiently obtained following the local infusion into the seminal vesicle.

From the standpoint of the plasmin system, the relationship between essential hemospermia and allergic seminal vesiculitis as well as the hemostatic efficacy of the plasmin inhibitor were discussed.

Introduction

Hemospermia has been defined as a syndrome in which the blood component is found in the seminal fluid. Twenty-four cases with hemospermia were statistically collected in our department, which occupied about 0.3 % of the male outpatients during the years from 1955 to 1964[1] With the knowledge about male sterility gradually increasing, there has been an increase of semen examinations in the urological department. Consequently, the discovery of hemospermia is gradually increasing. Because of this syndrome, fear of sterility causes psycological anxiety, especially effecting the young. This, therefore, is one of the very important reasons for a urologist to make a study on its etiology.

Since the etiology of hemospermia was first reported by Rapin[2] in 1859, several studies have been made. Organic hemospermia has been classified as one having clear clinical features, whereas essential hemospermia one clinical features of which are not clearly known. The latter is more prominent than the former. A few researchers have made clinical and experimental study of essential hemospermia. According to their studies, it was considered that the allergic reaction in the seminal vesicles is related with essential hemospermia. These concepts have been generally accepted. Along with an advance in

study of the fibrinolytic enzyme system, fibrinolysis has been confirmed as a very important subject in relation to urological diseases, especially concerning those of the kidney and prostate.

In the author's department, the studies of the fibrinolytic enzyme in the prostatic tumors have been made by Kuroda, Hisazumi and Korai[3] (1960). They have performed in remarkable detail the study of the tissue activator of plasminogen in the prostatic tumors. According to the studies by Kuroda et al.[4~6], Hisazumi and Iwasa[7] (1965) and Hisazumi (1968)[8], it is known that local fibrinolysis plays a very important role in postoperative bleeding into the prostatic cavity formed after prostatectomy, in comparison with general fibrinolysis. In addition, they have emphasized that local fibrinolysis often results in the bleeding of the genitourinary organs and others. Furthermore, Hisazumi and Fukushima[9] (1965), and Hisazumi[10] (1969) stated that, in the cases of hemospermia, the fibrinolytic activity of the seminal fluid of these patients showed a significantly higher level than that of normal men. They also suggested that the enhanced state of fibrinolytic activity in the seminal vesicles might be caused by an allergic reaction in the vesicles.

The purpose of the present investigation is to elucidate the relationships of the hemospermia with the allergic reaction in the seminal vesicles by means of the experimental study on fibrinolytic enzyme system.

Review of References

Since Rapin (1859) reported on hemospermia, there have been many works on the etiology of this syndrome. Ricord[11] believed it was caused by bleeding from the testis or epididymis. Nelaton[12] related it to bleeding caused by urethral injury. Ulzmann[13] also related it to bleeding caused by injury of the prostatic urethra. These are now accepted as explanations of hemospermia, but not as causes. Some researchers studied the bleeding of the prostatic gland, but in recent years there has been renewed interest in hemorrhagic lesions in the seminal vesicles. Guelloit[14] classified this, according to the site of the bleeding, into false and true hemospermia. In the former the urethra was regarded as the principal source of bleeding and in the latter, the seminal vesicles.

According to the opinion of Huggins and McDonald[15] (1945), in cases of prostatic bleeding the semen revealed uniformly mingled blood clots, whereas in seminal vesicle bleeding it appeared to be uniformly mixed turbid pink. They have, furthermore, tried to classify it as acute or chronic hemospermia in relation to the symptoms. Momose[16] (1961) and Endo[17] (1963) have etiologically classified hemospermia into organic and essential entities. Yata[18] (1963) regarded essential hemospermia as being due to a functional disturbance of the seminal vesicles.

Concerning inflammatory diseases, much attention has been paid to non-specific, tuberculous, and syphilitic inflammation. Nelken[19] (1910), Parker[20] (1942), Eisendrath and Polnik[21] emphasized the possibility of genital tuberculosis as an etiologic factor, but Lydston[22] (1894) and Huggins disputed this. In Japan, Inada[23] (1949) reported that hemospermia often appeared as an early symptom of tuberculous prostatitis. Sporer and Oppenheimer[24] (1957) expressed the same opinion. Some cases of hemospermia caused by tuberculous seminal vesiculitis were reported by Parker[20] and Namiki[25] (1960). In the latter's report,

the case showed calcification of the seminal vesicles originating from tuberculous inflammation.

Tomikawa[26] (1939) observed the presence of red blood cells in the seminal vesicle fluid obtained by catheterization to the ejaculatory ducts of patients with tuberculous epididymitis.

Kuroda[27] (1949) examined the semen of 24 patients suffering from genital tuberculosis, but there was no hemospermia. Ichikawa et al.[28] (1944) performed a vesiculectomy on patients with tuberculous seminal vesiculitis, but these specimens offered no evidence to support the presence of hemospermia. Thirty patients with hemospermia treated by Endo, 11 of whom had a past-history of pulmonary tuberculosis, showed a very low incidence of hemospermia caused by genital tuberculosis. A case of hemospermia reported by Arakawa et al.[29] (1944) showed syphilitic ulceration and a gumma in the seminal vesicles. Nelken also pointed out the important relationship between syphilis and hemospermia. Many cases of hemospermia caused by non-specific seminal vesiculitis as well as a few cases of non-specific prostatitis or posterior urethritis have been reported.

Tomikawa examined 51 specimens obtained from the cannulation of the ejaculatory ducts and found 9 specimens containing red blood cells of which 3 cases suffered from chronic gonorrhea and 3 chronic gonorrhea accompanied by prostatitis. Nakao[30] (1950) examining 4 patients with hemospermia found 3 cases with vesiculitis as an etiological disease. Ochiai et al.[31] (1959) removed the seminal vesicles from 2 patients with hemospermia under the presumptive diagnosis of chronic seminal vesiculitis, and found no special histological findings able to explain hemospermia. Kusunoki[32] (1947), Shimoe[33] (1959), Kanazawa et al.[34] (1960), Nagata et al.[35] (1961) and Momose et al. reported their own cases of hemospermia caused by diverticulum of the seminal vesicles, and concluded this disease might easily be diagnosed by seminal vesiculography. Although the diagnosis of diverticulum of the seminal vesicles is comparatively easy, that of seminal vesicle cyst is not so and, unfortunately, most frequently it accompanies hemospermia. In Europe, Stewart et al.[36] (1949) encountered one case of the seminal vesicles cyst with hemospermia and observed the cyst full of blood. In Japan, Ishigami[37] (1953), Nakamura et al.[38] (1955), and Momose et al. reported the similar cases. Magid and Hejtmanick[39] (1957) encountered 2 cases of hemospermia with direct communication between the pelvic vein plexus and the vesicular cavity. Endo, by injecting indigo carmine and Evans blue into the seminal vesicles, confirmed the transmission of these dyes into the blood but there was no evidence of hemospermia in connection with the above-metioned communications.

The etiology of organic hemospermia has been thought to be mostly the disease of the seminal vesicles and this concept is widely accepted nowadays. Paul and Cohn[40] (1907) observed that the submucosa of the seminal vesicles is quite vascular and tends to a bleeding condition. Lydston and Leader[41] (1958) agreed this. Masunaga[42] (1968) observed vessels penetrating vertically from the muscle layer to the submucous layer. These vessels were always in a condition of being pressed by the vesicular fluid ; and in the case of bladder neck disease, the ejaculatory duct was deformed resulting in the elevated seminal vesicle pressure which causes the rupture of the

vessels. Concerning other organic factors, Parker pointed out the significance of spermatocele as a cause of hemospermia. On the contrary, Ishigami[43] (1957) could not find any relationship between these diseases, and a low incidence of their co-existence was stressed. Omori et al.[44] (1953) and Kamimura et al.[45] (1954) reported a case of hemospermia associated with sperm invasion caused by injury of the epididymis and assumed an etiology of the hemospermia based on the inflammation and necrosis of the epididymis or the increase of the permeability of the vessels by the sperm hyaluronidase.

On the other hand, functional factors, as Yata had previously mentioned, such as allergy, sexual neurosis, sexual excess, over-masturbation and hemorrhagic diathesis were also discussed (Table 1). Lydston wrote that sexual excitation was the main reason for the congestion of the mucosa of the seminal vesicles. Parker thought that over-masturbation or sexual excess was the cause. Leader connected it with dissatisfaction of sexual desire. Furthermore, Tomikawa inserted a catheter into the seminal vesicles of the patients suffering from sexual neurasthenia in order to collect the vesicular fluid, 2 cases of which revealed a certain amount of red cells. With these results, he emphasized that the functional factors have some relation with hemospermia. However, Huggins remarked that the quantity of sexual activity or any sense of sexual emotion did not prove to have a connection with the occurrence of hemospermia.

If allergy has any concern with this syndrome is still not clear.

Nakao classified 2 types of syndrome: those with clear organic causes were called "hemospermia", but others were called "bloody seminal syndrome". Nelken, however, named the latter "essential hemospermia", to handle it as an independent diseases. From the histological point of view, Ishigami ascertained the marked dilatation or disruption of the vessels with no inflammatory changes being seen, and, furthermore, infered this to be essential seminal vesicle bleeding. Likewise, Ochiai et al. also confronted with the same cases, but found no specific changes from the histological sections. Cases of seminal vesicle cysts reported by Nakajima et al.[46] (1958) and of diverticulum of the seminal vesicles by Kanazawa et al. showed a slight infiltration of round cells in the mucosa but no evidence of bleeding. Thus, the cause of the bleeding remains unclear. Pastinszky[47] (1959) related allergic seminal vesiculitis of the male genital organ system to hemospermia and eosinophils in the tissue. Nagata et al. observed edema and a large

Table 1 Etiological Classification of Hemospermia (by Yata, B., 1963)

I) Hemospermia due to organic disturbances (Seminal tract)
1) Anatomical and morphological abnomalities
a) Pathological dilatation of the seminal vesicles and the vas deferens
b) Direct communication between the venous system and the seminal vesicles
2) Non-specific inflammation
a) Bacterial inflammation
b) Aseptic inflammation
3) Specific inflammation
a) Tuberculous inflammation
b) Syphilitic inflammation
4) Stones
5) Tumors
6) Traumas
II) Hemospermia due to functional disturbances
1) Allergy
2) Sexual neurasthenia
3) Sexual excess or gross masturbation
4) Hemorrhagic diathesis

amount of eosinophils in the submucous layer but without a specific infectious picture. Momose, however, considered focal infection and allergy as the causes of hemospermia, and postulated essential hemospermia as having no clear cause. Because anti-allergic drugs proved therapeutic effect for essential hemospermia, Yata made an experimental allergic reactions in the seminal vesicles of the rabbits by using a filtrate of E. coli culture medium as an antigen and histologically ascertained the bleeding of the mucosa and submucosa with necrosis, edema and infiltration of round cells as well as the changes in the wall of the vessels. Because these findings were common to essential hemospermia, he emphasized the presence of allergic reactions. Endo examined in detail 30 patients with hemospermia, some of which were suspected of having essential hemospermia. In these cases eosinophilia and vagotonia were evident and the administration of anti-allergic drugs was shown to be effective. Furthermore, the majority of these patients had a past history of urethritis. Consequently, he supposed infectious allergy has played a certain role in hemospermia. In allergic seminal vesiculitis of the rabbits caused by injecting pig serum, he observed a reaction resembling essential hemospermia. He then believed one of the causes of hemospermia was an antigen-antibody reaction due to foreign protein. Masunaga, from the clinical point of view, regarded allergy as an important cause of hemospermia.

In the author's department, 10 cases of essential hemospermia were closely examined by Hisazumi. From the view of the fibrinolytic enzyme system, he made examination of the semens of these patients, comparing them with those of normal adults

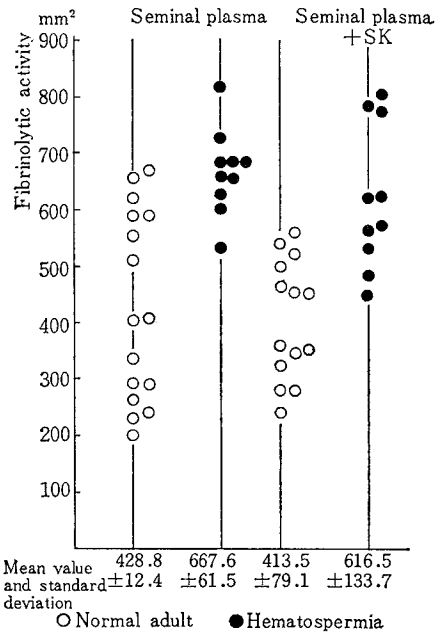


Fig. 1. Fibrinolytic activity of seminal plasma.

(Fig. 1). This study showed with a statistical significance higher level of fibrinolytic activity of the seminal fluid of the patients than the normal group, leading him to conclude that essential hemospermia results from allergic reactions. Moreover, he proposed that epsilon-aminocaproic acid is a very useful hemostatic agent in essential hemospermia.

Rochea e Silva[48] (1943) proved that the antigen-antibody reaction activated the fibrinolytic enzyme in the blood. Hitherto, there have been many works on this concept[49~51] Ungar[52,53] (1947) made an experimental allergy using slices of the lung, liver and kidney of the sensitized guinea pigs and by adding the antigen to vitreum. As a result of this allergic reaction activated fibrinolytic enzyme was noted. In recent years, clinical and experimental research has shown that at the sites of allergic reaction, i.e. the skin[54], joints[55~57], liver[58] and kidney[59] etc., there is an elevation of fibrinolytic activity.

Fleischer[60] (1915) started the research of the local fibrinolytic enzyme system. Astrup and Permin[61] (1947) obtained the activator of plasminogen from the extraction of human tissues, which was known as a tissue activator. Estimation of the activity of the tissue activator was described by Astrup and Albrechtsen[62] (1957). The tissue activator has been extracted by 2 M KSCN, 0.9% saline solution and KCl solution as a solvent, and generally the activity level has been calculated by the fibrin plate method. In the following year, Todd[63] (1958) described the topographical method, in which a frozen section from the tongue, muscle, lung, spleen, kidney, adrenal gland, uterus, prostate and aorta, is covered by the fibrin membrane, and incubated at 37°C, then fixed and stained. The dissolved area of the fibrin membrane indicated the presence of a tissue activator. According to Todd[64~66], the localization of the tissue activator was confirmed to be great in the endothelial cells of the small veins in all organs. However, in the artery, the tissue activator was only slightly present or completely absent. In addition, the SK-added fibrin membrane showed an increase of the lyzed zone, indicating the presence of a proactivator in the vessels. Likewise, Kwaan et al.[67,68] (1963) and other researchers[69~74] modified this method, and reported that only the small vein revealed the lyzed zone. In Japan, Soma[75] (1963) first carried out this method, and observed the lyzed zone around the area of the vessels.

In regard to tissue lesion, Prokowicz[76] provoked damage of the kidney (1964) by administering mercury perchloride to the dog and first observed the tissue activator by the topographical method. In Japan, Sugiura[77] (1968) applied this method to a section of prostatic cancer. Yamamoto[78] (1966) used the experimental liver fibrosis. Horiuchi[79,80] (1968) induced local arthritis in a dog by using turpentine oil. Kawai et al.[81,82] (1968) injected nephrotoxin into the rabbit to obtain a nephritis specimen. All of these tissues were histochemically observed to determine the localization of the tissue activator, and each of these showed an increase of fibrinolytic activity compared with the normal group. In the normal liver no lyzed zone could be usually found, but there was a surprising amount of tissue activator in some normal organs. Therefore, compared with the abnormal organs, the determination of tissue activator activity of the normal organs is very difficult. Kwaan[83] determined the level of the tissue activator by means of focal lysis time and by shortening the incubation time. He also semiquantitatively determined the potency by converting the focal lysis time to the unit of the urokinase concentration. Pandolfi[84] (1967) classified the degree of the lyzed zone into three grades. Grade 1 indicated the lyzed zone observed only by microscope, grade 2 by the naked eye, grade 3 complete lysis of the fibrin membrane. One mark stood for grade 1, two marks for grade 2 and so on. The degree of the lyzed zone for the different incubation periods such as 0, 5, 10, and 20 minutes was accounted by means of the total number of marks obtained.

The relationship between hemospermia and pregnancy were discussed by Mitsuya[85] (1949). The hemospermia patients showed the least amount of fructose in the semen. By this observation, he emphasized the important relationship between the presence of blood and the level of fructose in the semen. Arakawa (1944) pointed out that the hemospermia followed by chronic infection was accompanied by oligozoosper-

mia. Other discussions concerning this syndrome were mainly focused on azoospermia, teratozoospermia, low motility of sperm, and etc. Endo examined the hemospermia patients, but found neither impaired sperm motility nor change of fructose in the semen, and concluded that there were no bad effects on fertility of the sperm.

Materials and Methods

1. Animal experiments.

72 adult male rabbits, weighing above 2 kg, were used for this study. A control group was also made of 25 rabbits.

2. Preparation of fibrinogen.

Bovine fibrinogen was obtained by the following procedure:

Fresh blood containing 0.1 N sodium oxalate was centrifuged, and solid barium sulfate was added at room temperature to the plasma obtained. In this way, prothrombin and the clotting factor (factor VII) were absorbed. To this clear solution one-third volume of saturated ammonium sulfate was added at 4°C. The obtained precipitate was dissolved in 0.9 % saline solution. This procedure was performed repeatedly for 3 times in a cold room. The purified fibrinogen obtained was dissolved in veronal buffer[86,87] (pH 7.6, μ=0.15).

Human fibrinogen (Midori-Juji Co. product) was dissolved in 0.1 M phosphate buffer (pH 7.4).

Rabbit fibrinogen prepared from fresh heart blood using the above-mentioned procedure was dissolved in veronal buffer.

3. Thrombin (Park-Davis product) was dissolved in 0.9 % saline solution (20 units /ml).

4. Streptokinase (SK) (Varidase, Lederle product) was dissolved in 0.9 % saline solution (10 units/ml).

5. Antigen (egg albumin, Nutritional Biochemical Co.) was dissolved in 0.9 % saline solution (10 mg/ml). One milliliter of this solution was added to a mixture of 0.85 ml of liquid paraffin, 0.5 ml of Arlacel and 5 mg of BCG[88]. In this way, an antigen was readily obtained.

6. Evaluation of plasmin activity in blood.

The estimation was made before and after the allergic reaction occurred in the seminal vesicles of the rabbits. The fresh blood was obtained by means of a silicon tube inserted into the heart and connected with a syringe. Five milliliter of blood was then mixed with 0.5 ml of 0.1 N sodium oxalate to obtain plasma. One milliliter of this plasma was diluted 10-fold with cold distilled water and then acidified to pH 5.2 by about 1 ml of 1 % acetic acid. It was then left standing for 1 hour at 4°C until the solution precipitated, and then centrifuged at 4°C and 3,000 r.p.m. for 10 minutes. The precipitate was dissolved in the phosphate buffer to the original volume of plasma (pH 7.4)[89] This was used as euglobulin solution and 0.03 ml of this solution was dropped on the surface of standard fibrin plates which were prepared by adding 0.2 ml of thrombin (20 units/ml) to 8 ml of 0.1 % bovine fibrinogen solution in a Petri dish. The plate was then incubated at 37° C for 18 hours. The activity was recorded as the diameter products in sq. mm of the lyzed zones. To obtain the whole activity of the plasmin, SK solution was added (10 units/ml, 0.03 ml) to each drop of euglobulin and incubated in the same condition.

7. Preparation of allergic reaction in the seminal vesicles.

This was prepared by the complete adjuvant method which was described by Freund[90] (1942). Egg albumin was used as antigen. Antigen of 5 mg per kg body

weight was injected intramuscularly into the back once a week for 2 weeks, and 3 weeks after the last injection was performed, the antibody titer was estimated by the ring test. In this experiment, a rising of the antibody titer was sufficient. A 0.5 ml of antigen (egg albumin 10 mg/ml) was injected through both vas deferens into the seminal vesicles of each rabbit, the capacity of which was about 1 ml.[91] Vesiculectomy was performed after 24 hours, and the seminal vesicle fluid was concomitantly collected.

8. Estimation of tissue activator.

This was performed according to the Astrup and Albrechtsen's method (1957).

The removed seminal vesicles were divided uniformly into 2 parts, and weighed. To one part a 20-fold solution of 2 M KSCN was added, homogenized for 10 minutes, and then shaken for 60 minutes. It was then centrifuged at 9,000×g for 10 minutes. To 1 ml of this supernatant, 7 ml of cold distilled water was added and acidified to pH 1.0 by 1 N HCl. It was allowed to stand at 4°C for 30 minutes, and was then centrifuged again at 3,000 r.p.m. for 10 minutes. One milliliter of 2 M KSCN was added to the precipitate, neutralized and dissolved by sodium bicarbonate powder. To this solution 1 ml of veronal buffer was added and 0.03 ml of this mixture was applied on the surface of standard and heated fibrin plates. The latter plate had been prepared by heating at 85°C for 30 minutes, the purpose of which was to destroy plasminogen contaminating fibrinogen[92]. With this plate it could be determined whether plasmin existed in the applied sample or not. The activity of the tissue activator was recorded by using the method which was described in the 6th paragraph.

9. Histochemical determination of tissue activator (topographical method).

According to Todd's method (1958), the removed seminal vesicles were promptly frozen by liquid carbon dioxide, cut into 10 μ thickness using a cryostat (Cryocut, American Optical Co.) at −20°C, and then placed on a glass slide with a square line mark of 2.5×3.0 cm made by water proof ink. After thawing they were rapidly dried. Then 0.3 ml of 0.5 % fibrinogen solution (rabbit or human) was applied to the marked area and a drop of thrombin (20 units/ml) was added. It was then nimbly stirred so as to spread in a uniform thickness on a horizontally placed glass plate. Then the slide was kept for 30 minutes at 4°C. This caused the specimen to be covered by about a 0.4 mm thick fibrin membrane. This was then placed in a plastic case and incubated at 37°C, after which it was fixed in formalin gas for 30 minutes. It was then immersed into 0.9 % saline solution containing 10 % formalin for 30 minutes. After being washed, it was stained by Harris' hematoxylin and eosin. Before being microscopically examined, it was mounted with gelatin liquid and preserved for future reference. The frozen section was placed on the newly made fibrin membrane after it was heated for 30 minutes at 85°C. It was then incubated to determine whether the activity of plasmin existed or not. The removed seminal vesicles were also ordinarily sectioned and stained for the purpose of comparing these results.

10. Estimation of the fibrinolytic activity of seminal secretion.

The secretion of the removed seminal vesicles was centrifuged at 3,000r.p.m. for 15 minutes. By means of the above-mentioned method of standard and heated fibrin plates, the supernatant was used to determine the fibrinolytic activities. Also whole activity

of the plasmin was determined by the same method after an equal amount of SK solution (10 units/ml) was added to the obtained supernatant.

11. Trans-aminomethylcyclohexanecarboxylic acid (trans-AMCHA), (Transamin in 5 W/V % solution, Daiichi Seiyaku Co. product) was used.

12. Urokinase (Midori Juji Co. product) was dissolved in 0.9 % saline solution (2 units/ml).

13. Plasmin (Midori Juji Co. product) was dissolved phosphate buffer (pH 7.4, 0.5 units/ml).

Results

1. The effects of purified egg albumin on urokinase and plasmin.

In an attempt to observe the effects of egg albumin upon the fibrinolytic agents, the following experiments were carried out.

Aliquots of 1 ml of urokinase were placed in a series of test tubes, to each of which was added 1 ml of egg albumin solution in concentrations from 1.75 to 10 mg/ml. On the surface of standard fibrin plates 0.03 ml of these mixtures was dropped. Activities of these samples were recorded as the diameter products in sq. mm of the lyzed zones after 18 hours at 37°C. However, there were no effects of egg albumin on the urokinase.

Instead of urokinase, plasmin was used for the same experiment, and the mixture was placed on the surface of the heated plates. As shown in Fig. 2, the activities

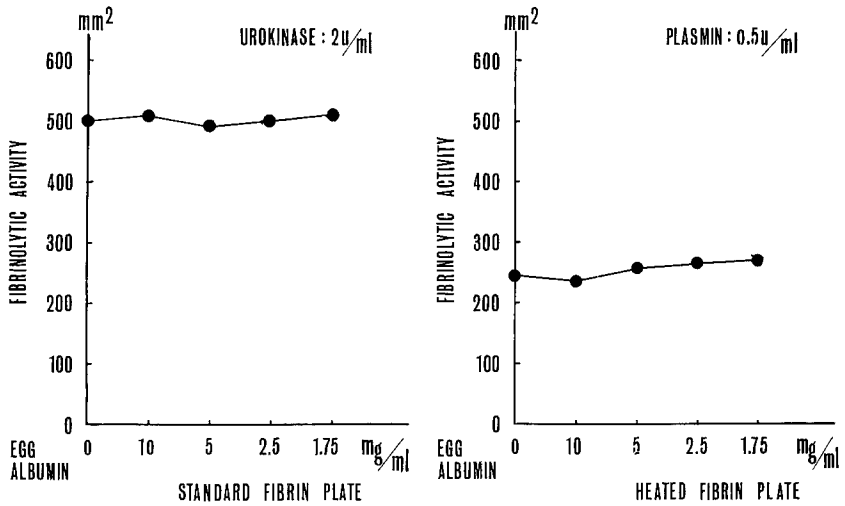


Fig. 2. Effects of egg albumin upon the activity of plasmin and urokinase.

of different concentrations of plasmin were not influenced by addition of egg albumin.

2. Estimation of antibody titer.

By the ring test, 28 rabbits were estimated in this experiment. As shown in Table 2, the antibody titers showed a range from 2^5 to 2^9 fold in different dilutions of antibody.

Table 2.

Antibody titer	No. of Rabbits
$\times 2^5$	1
$\times 2^6$	4
$\times 2^7$	8
$\times 2^8$	9
$\times 2^9$	6

3. The histological findings of the seminal vesicles after the local allergic reaction.

As shown in Fig. 3, the mucosa of the

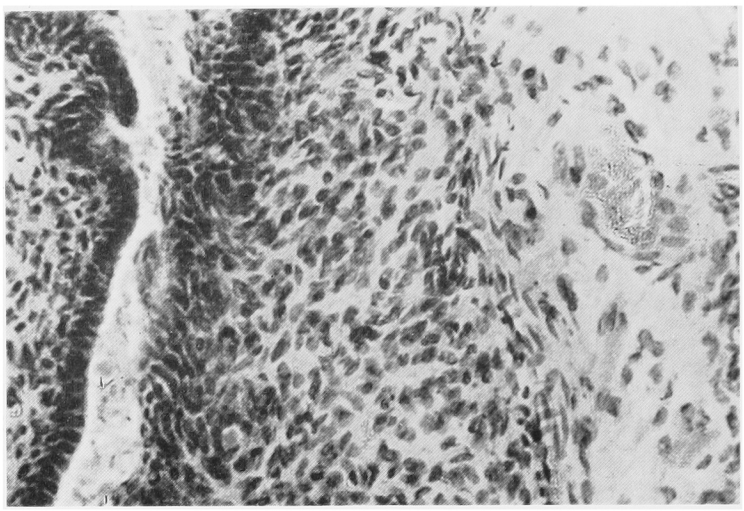


Fig. 3. Microscopic section of the seminal vesicles of a normal rabbit, H & E. ×400.

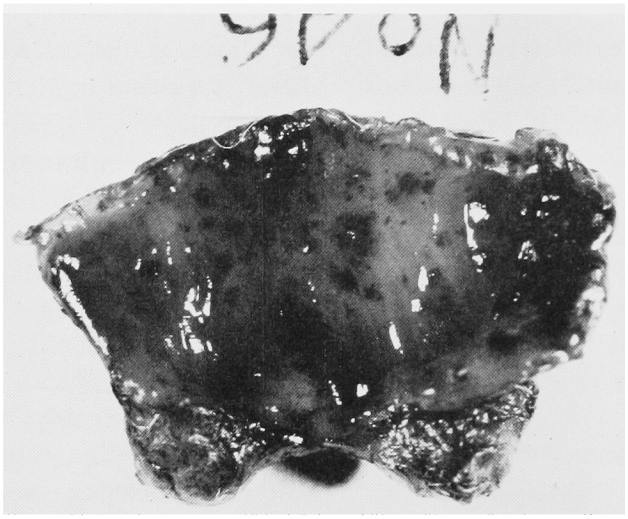


Fig. 4. Photograph of resected seminal vesicles after allergic reaction. Note submucous edema with multiple petechiae.

normal seminal vesicles essentially consisted of one layer of columnar epithelium with exception of the caudal portion having two layers. The submucosa consisted of connective tissue containing many small vessels and elastic fibers, and the muscular layer consisted of an inner longitudinal layer and an outer circular layer.

The seminal vesicles after the allergic reaction macroscopically revealed an edema of the inner surface showing agglomerated or disseminated petechiae (Fig. 4). In the microscopic examination, there was no change in the mucous epithelium, but the connective tissue of the submucous layer was primarily infiltrated by eosinophils and lymphocytes in a diffuse fashion. The majority of the small vessels showed dilatation and congestion causing petechia, and the connective fibers showed a loose arrangement owing to an extreme edema. The muscular layer also showed capillary con-

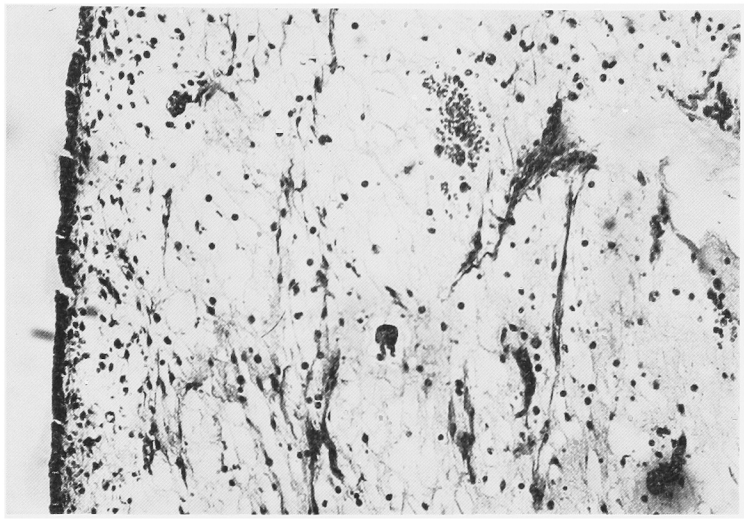


Fig. 5. Microscopic section of the seminal vesicles after allergic reaction. Note extreme submucous edema and hemorrhage, H & E. ×100.

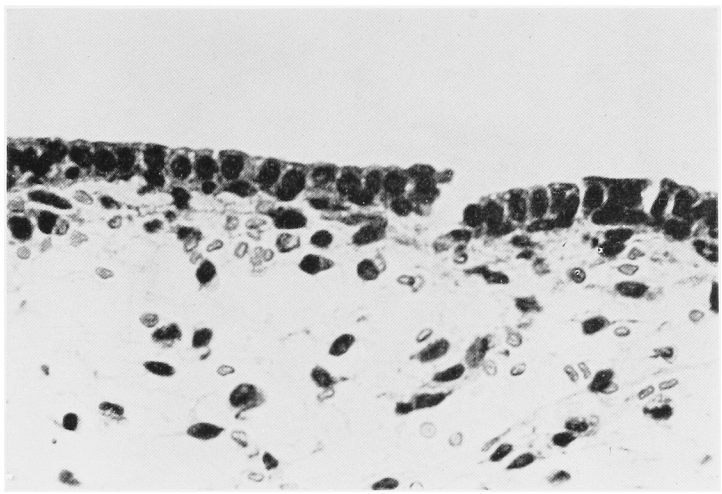


Fig. 6. Microscopic section of the seminal vesicles after allergic reaction. Note submucous edema and hemorrhage, H & E. ×400.

gestion accompanying small hemorrhages. The above findings coincided exactly with the allergic inflammation (Figs. 5 and 6). However, the degree of allergic inflammation did not always correlate with the titer of the serum antibody.

4. Plasmin activity in blood before and after the occurrence of allergic reactions in the seminal vesicles.

Of 45 rabbits in the allergic group, 18 were used for the estimation of the plasmin activity. In these experiments, the fibrinolytic activity of the euglobulin solution did not significantly appear on the fibrin plates. Thus, this was of little value in the estimation of the plasmin activity. For this reason, the SK-activated plasmin activity (whole activity) was determined. As shown in Fig. 7, there was an elevation of plasmin activity in 7 rabbits (above 50 % elevation in 3, above 20 % in 2 and below 10 % in 2) and a decrease in 10 (above 50 % decrease in 4, above 10 % in 3 and below 10 % in 3); and, in both groups, this was

estimated after the allergic reaction occurred. No difference of plasmin activity was observed in 1 rabbit.

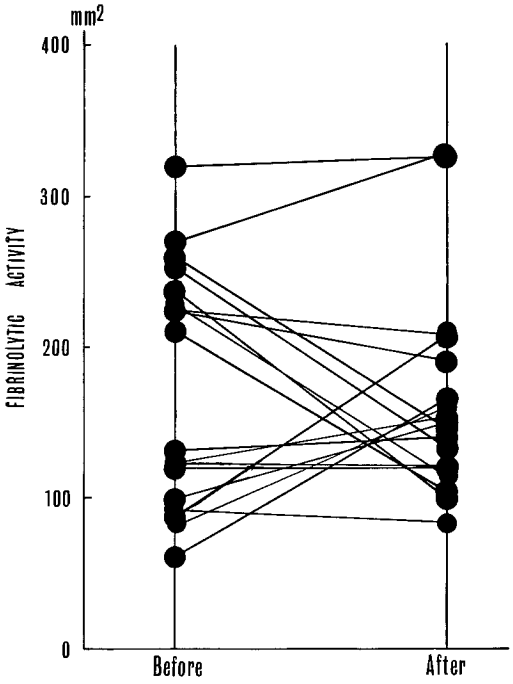


Fig. 7. Fibrinolytic activity in the circulating blood before and after allergic reaction.

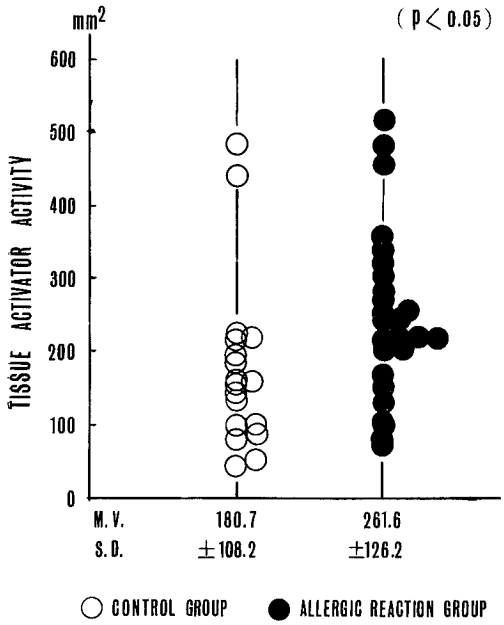


Fig. 8. Tissue activator activity (KSCN-extract).

Consequently, the statistical analysis of the plasmin level in the blood showed no significant difference between pre- and post-allergic reactions.

5. Estimation of tissue activator activity.

Twenty five rabbits were used as the control group for this experiment. As shown in Fig. 8, the value ranged from 53 to 483 mm², with a mean value of 180.6±standard error of 108.2 mm², whereas, in the allergic group of 28 rabbits, the value ranged from 82 to 517 mm², with a mean value of 261± standard error of 126.2 mm². Statistical analysis of these two groups revealed a highly significant difference ($P<0.05$). This means that the allergic group showed an increased tissue activator activity. There was no fibrinolytic activity on the heated plates in either group. Therefore, it was clear that the fibrinolytic activity was caused by the plasminogen activator and not by the non-specific proteolytic enzymes. However, there was no significant relationship between the value of the tissue activator activity and the titer of the serum antibody in the allergic group (Fig. 9). Moreover,

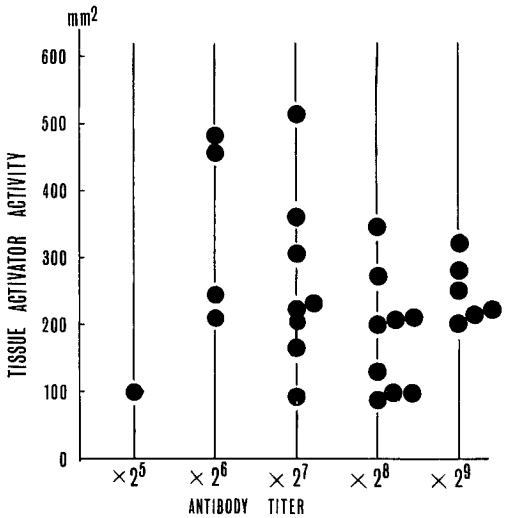


Fig. 9. Comparison between tissue activator activity and antibody titer.

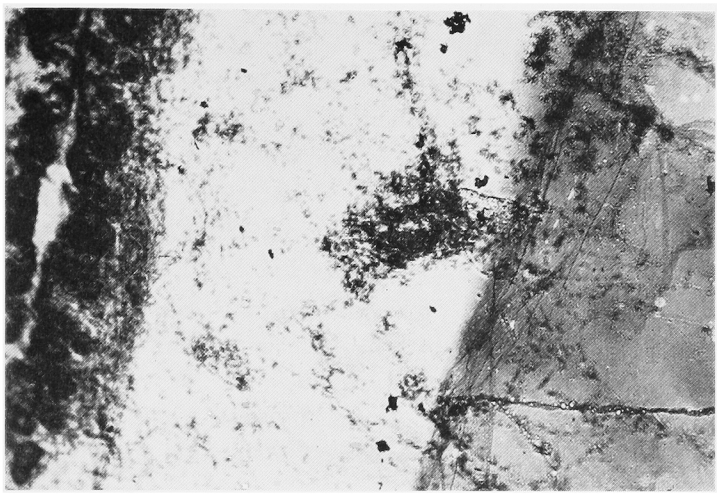


Fig. 10.
Fibrinolysis autograph of seminal vesicle tissue showing striking fibrinolytic activity after allergic reaction.
Note that, when using rabbit fibrin, focal lysis occurs at the submucous layer after 20 minutes incubation. ×100.

Fig. 11.
Rabbit fibrin membrane preparation showing a section with allergic reactions surrounded by a zone of lysis, 60 minutes incubation time. ×40.

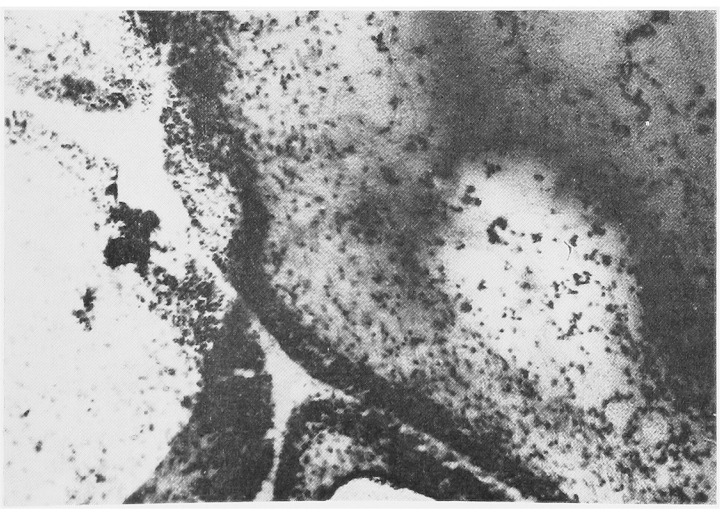


Fig. 12.
Human fibrin membrane preparation showing focal lysis at the submucous layer after allergic reaction, 90 minutes after incubation. ×100.

the activity of the tissue activator was not always parallel to the degree of allergic reaction in the seminal vesicles.

6. Topographical observation of tissue activator (Todd's method).

In the case of the rabbit fibrin membrane, the focal lysis occurred as a clear zone at the site of the allergic reactions in the seminal vesicles revealing edema, congestion, petechia and the infiltration of eosinophils, 20 minutes after incubation at 37°C (Fig. 10). Furthermore, the zone gradually spread after 30 minutes of incubation, and, after 60 minutes, the fibrin membrane completely dissolved (Fig. 11). When the human fibrin membrane was used, a clear zone of focal lysis appeared by 90 minutes (Fig. 12), whereas in the control group no focal lysis could be observed until 90 minutes of incubation, when a slight focal lysis was observed in the capillary network of the submucosa. No focal lysis was observed until 2 hours of incubation when human fibrin was used.

These results indicated that there was a shortening of the "focal lysis time" in the

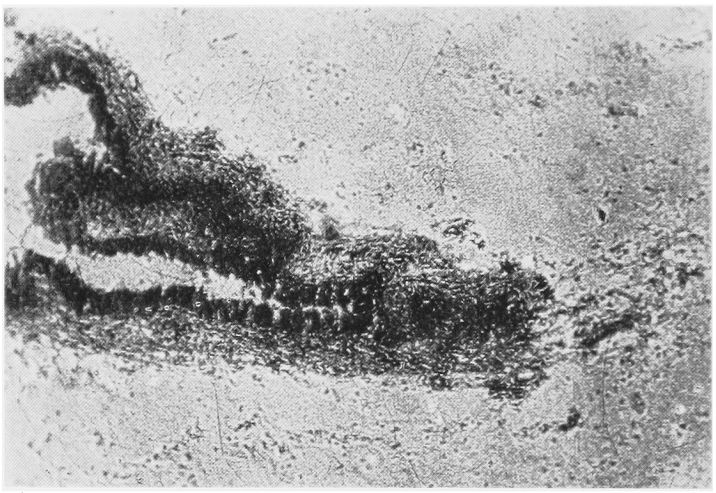
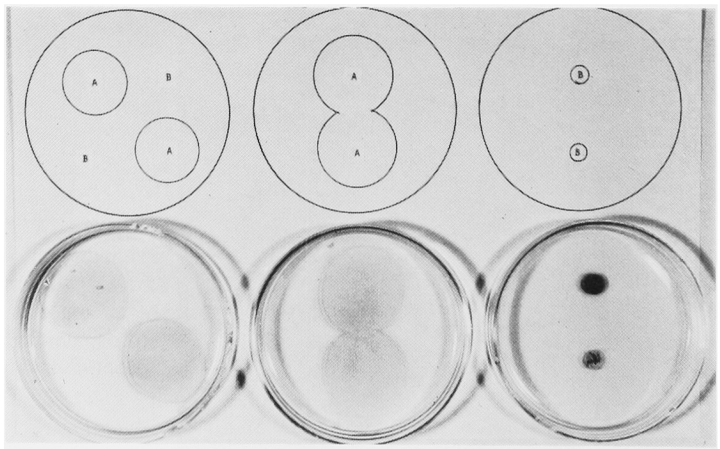


Fig. 13. When using heated rabbit fibrin in the allergic tissue of the seminal vesicles, there was no focal lysis, even after 120 minutes of incubation. ×100.



Seminal vesicle secretion Seminal vesicle secretion+SK

Fig. 14. Fibrinolytic activity of seminal vesicle secretion on standard fibrin plates: A) rabbit No. 48 (allergic group), B) rabbit No. 50 (control group).

allergic group.

When the heated fibrin membrane of both were used, no focal lysis was observed in either group even after prolonged incubation (Fig. 13). These results indicated that the focal lysis was caused by the plasminogen activator.

In our experiments, we could not confirm the connection between the focal lysis and the small veins containing a considerable amount of tissue activator as described by Todd.

The bovine membrane showed no focal lysis in any condition.

7. Fibrinolytic activity of the seminal vesicle secretion.

When the standard fibrin plates were used, the estimation of the activity of the control group (10 rabbits) was very difficult, because the seminal vesicle secretion lyzed only partially the surface of the fibrin plate (Fig. 14). The allergic group (10 rabbits), however, showed a value ranging from 25 to 1,295 mm^2. Concerning the seminal vesicle secretion to which SK was added (10 units), as shown in Figs. 14, 15, the control group showed the highest value with 80 mm^2, but the allergic group showed a value ranging from 84 to 1,443 mm^2

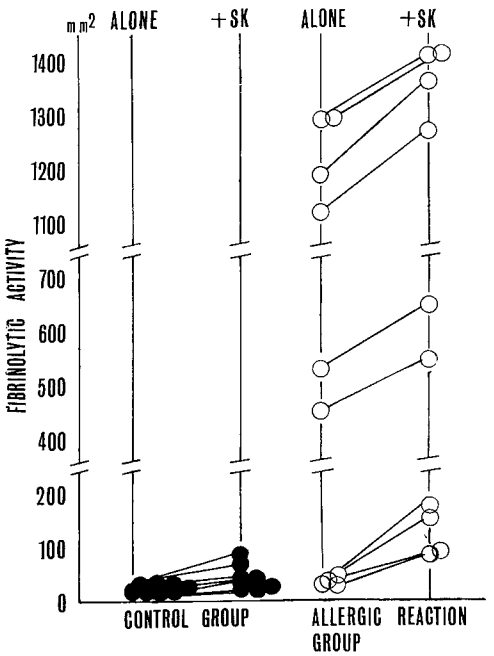


Fig. 15. Fibrinolytic activity of seminal vesicle secretion.

When heated fibrin plates were used, both group showed no fibrinolytic activity.

These results proved a more significant elevation of fibrinolytic activity at the site of local allergic reactions than those obtained from the KSCN extraction method or the topographical method. Furthermore, these results show not only an increase of plasminogen activator, but also, when SK solution is added, an increase of proactivator and/or plasminogen.

8. The efficacy of drug treatment[93] (trans-AMCHA).

a) General administration group

Thirty minutes before the antigen was injected, 250 mg per kg of body weight of trans-AMCHA was injected into the auricular veins of 8 of 47 rabbits in the allergic group.

b) Local administration group

Separate syringes of antigen and 10 mg/ml of trans-AMCHA were simultaneously injected via the vas deferens to the seminal vesicles of 8 of 47 rabbits in the allergic group.

Histological findings :

No suppression of the inflammation could be observed in the group a). Furthermore, as in the unadministered group, there were edema, congestion, petechia and the infiltration of eosinophils.

The group b) showed the same results, but, compared with the unadministered group, had a slight suppression of inflammation.

Estimation of the activity of the tissue activator (Fig. 16) :

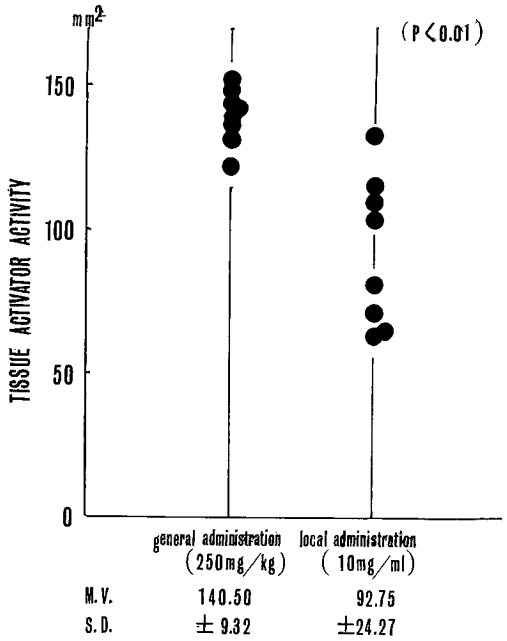


Fig. 16. Tissue activator activity in allergic group with trans-AMCHA administration.

The group a) showed a value ranging from 121 to 125 mm², and had a mean value of 140.50±standard error of the mean of 24.27 mm². The group b) showed a value ranging from 63 to 110 mm², and had a mean value of 92±standard error of the mean of 9.32 mm². Both the groups a) and b) showed a significantly lower value than the unadministered allergic group. The group b), moreover, showed a significantly lower value than the group a). Naturally, the results might be dependent not only on the quantity of trans-AMCHA administered, but on method of administration just as the local administration of it gave a better suppressive effect than the general administration.

Topographical observation of the tissue activator :

When 0.4 % of human fibrin membrane was used for 120 and 150 minutes of incubation for groups a) and b) respectively, focal lysis appeared after a comparatively longer time than that of the unadministered allergic group.

The fibrinolytic activity of the seminal vesicle secretion (Fig. 17) :

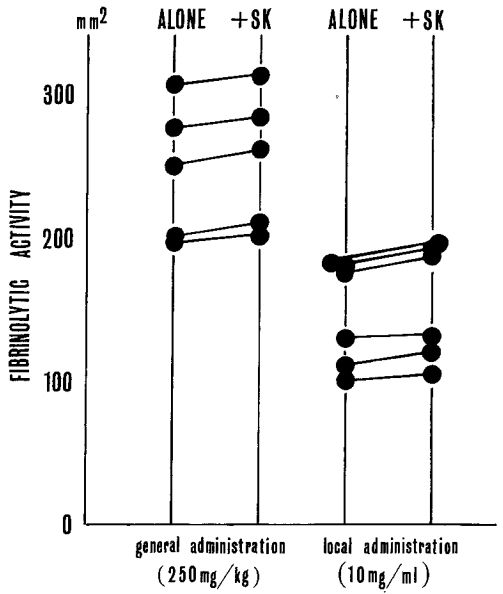


Fig. 17. Fibrinolytic activity of seminal vesicle secretion in allergic group with trans-AMCHA administration.

In both the groups a) and b), compared with unadministered allergic group, the activity was depressed, but the group b) showed a significantly lower value than the group a). These results were similar to the results obtained from the KSCN extraction method.

Discussion

According to the present study of allergic reactions in the seminal vesicles, all cases revealed edema, vascular dilatation, congestion, petechia and infiltration of eosinophils in the submucosa, and these observations corresponded to the clinical and experimental findings of Endo and Yata. Endo made the sensitization by intravenous injection of antigen, which produced the immediate (Arthus) type of allergic reaction. In my research, the complete adjuvant

method was used, and the allergic reaction in the seminal vesicles was induced during a highly sensitized period. The findings of these tissue sections closely resembled the changes in the Arthus' phenomenon.

Concerning the study on the correlation of allergic reactions and fibrinolytic activity of the blood, Amemiya[94] (1960) reported that patients with drug eruptions often had an elevation of plasmin activity, and Minami[95] (1966) also reported an elevation of fibrinolytic activity in the blood of patients with autosensitized dermatitis, drug eruption or toxicoderma. Kawai (1968) viewed that there was a tendency for the plasmin activity to slightly increase under experimental nephritis in rabbits. When Igai (1967) made artificial arthritis in rabbits, there was not only an elevation of fibrinolytic activity of the blood, but a high level of antiplasmin. Kimura[96] (1964), studying the fibrinolytic activity of the ocular chamber fluid, injected human plasma into the ocular chamber of rabbits. He expected an elevation of both the local fibrinolytic activity and plasmin activity, but an inhibition of plasmin activity occurred. According to my experimental study, the influence of local allergic reactions upon the general fibrinolysis was not observed, and these results were identical with Hisazumi and Fukushima (1965), whereas, Shigematsu[97] (1964) and Suzuki[98] (1964) reported a derivation of general fibrinolysis from local fibrinolysis due to the release of tissue activator into the circulating blood. The seminal vesicles have both secreting and absorbing function[99,100], the latter of which could be shown by the santonin test. Regarding these concepts, the possibility of activation of the fibrinolytic enzyme in the blood by the tissue activator must be considered. It is logical to understand that local fibrinolysis is an important factor in the cause of hemospermia and, if there is general fibrinolysis concomitantly, it is merely a secondary phenomenon which might be influenced by local fibrinolysis.

Abe[101] (1961) injected turpentine oil into the hip muscle of a dog to induce aseptic inflammation. He checked the activity of the tissue activator and found that the activity of the tissue activator gradually increased, follwing the wide spread of the inflammation. Concurrently, the production of plasmin and plasminogen in the tissue were assured. Hatano[102] (1963) made experimental dermatitis in the guinea pig by 2-dimethylchlorbenzen, and observed the elevated tissue activator activity in the inflamed skin. Hayashi[103,104] (1965) brought about the Arthus' phenomenon in the skin of the rabbit and observed a strikingly elevated activity of the proteolytic enzymes in the euglobulin fraction obtained from the homogenized tissue at the most severe state of inflammation. Local allergic reactions in my experiment closely resemble the histological findings of the Arthus' phenomenon and accompanied increased tissue activator activity. Raab et al.[105] (1965), making the immediate and delayed types of allergic reaction in guinea pigs, found an increase in the proteolytic enzyme activity without a significant difference between both types. So to speak, the increase of the local fibrinolytic activity has no relationship with any type of allergic reaction. The author's experimental results showed no significant relation between the titer of serum antibody or the degree of allergic reaction and the tissue activator activity. Furthermore, the distribution of the tissue activator activity in the normal group was wide in range with the remarkable individual difference.

Considering the localization of the tissue

activator, we applied the topographical method which has been described by Todd. In all the cases, the focal lysis was observed at the submucosa after the allergic reaction occurred. However, where the initial site of the focal lysis in the histological level could not be elucidated. Even by changing the incubation time or the concentration of fibrin, it remained unclear. Thus, it was impossible to detect the localization of the tissue activator by any means.

When the focal lysis at the submucosa was present, that at the muscle and serosa was completely absent. However, when the lysis extended entirely over the tissue section, it was believed that the tissue activators contained in the muscle and serosa were related to the lysis. At the submucosa of the seminal vesicles there were numerous small vessels besides the allergic reactions. Thus, it was conjectured that the focal lysis was suspected to occur firstly in the endothelium of the vessel wall and each small lyzed zone could be viewed, being clouded together, as only one fused lyzed zone. Moreover, damage to the wall of the vessels occurred because of the allergic reactions. Spreading of the tissue activator made it difficult to confirm the site of the focal lysis in the histological level. Consequently, whether the eosinophils, neutrophils and lymphocytes were related to the fibrinolytic activity or not is not known. Rulot[106] (1904) discovered the "fibrin-digestive enzyme" in the white blood cells of horses. Opie[107] (1907) observed the activated proteases of polymorphonucleocytes (leucoprotease) and monocytes (lymphoprotease) in an alkaline side from the fibrinous exudate of dogs. Astrup et al.[108] (1967) determined the fibrinolytic activity of blood cells, by making a human blood smear applying the fibrin slide method and fibrin plate method. Normal leucocytes showed no fibrin film lysis, but the damaged leucocytes showed moderate focal lysis. Furthermore, the damaged leucocytes showed the focal lysis by using the plasminogen-free fibrin in the same degree with lysis of plasminogen-rich fibrin. The results indicated that there were non-specific proteolytic enzymes in the leucocytes. Barnhart and Riddle[109] (1963) studied the localization of plasminogen in the acidophil granules of the bone marrow by applying the fluorescent antibody method, and observed that the eosinophils were full of antiplasmin and that plasminogen was also present more abundantly in the more immature eosinophils. The eosinophils of bone marrow have the ability to produce plasminogen, which is transported into the circulating blood, consequently being released into the tissue. Prokowicz and Stormorken[110] (1968) used the fibrin slide method and confirmed that both granulocytes and monocytes provided fibrinolytic enzymes, but not lymphocytes, and the amount of the enzymes was equal in both eosinophils and neutrophils. From the works of the above researchers, it is quite clear that the infiltration of eosinophils and neutrophils on the allergic reactions plays an important role in focal lysis, but, at present, it is still hard to prove this concept.

In this study, the fibrin film method was conducted, using rabbit and human fibrins, and it was found that the focal lysis time of these two showed no significant difference at all. However, Warren[111] (1968) carried out this experiment by the histochemical method and, investigating the fibrinolytic activity of the rabbit's vena cava, could not observe the focal lysis by using the bovine fibrin film; but, when the rabbit fibrin film was used the focal lysis

appeared. It was, therefore, concluded that this reaction has a species specificity. Furthermore, Onoyama[112] (1966), applying the topographical method using the intima of the aorta, tried the fibrin film of bovines, humans and rabbits for comparing the intensity of the focal lysis, and found the rabbit fibrin to be most intensive while human fibrin was next. Similarly, he emphasized the species specificity of the activator. In this study the same results were obtained, and when the bovine fibrin film was used there was no focal lysis at all despite of the incubation of more than 6 hours. Whenever the low activity of the tissue activator of the rabbit was mentioned, it was a matter of comparison with bovine fibrin film.

Human semen contains a considerable amount of plasminogen activator, proactivator and antiplasmin[113] The prostate is said to contain a considerable amount of plasminogen activator. When 2 ml prostatic fluid was added to 100ml of human blood clot allowing to stand at 37°C for 18 hours, the clot dissolved. Huggins and McDonald confirmed that there was also abundant "fibrinolysin" in the seminal fluid, and Hisazumi ascertained the presence of the fibrinolytic enzyme in human seminal plasma and postulated that it has something to do with hemospermia. Experimental data of this paper showed that there is an exceedingly little amount of plasminogen activator and proactivator in the seminal vesicle secretion of normal rabbits compared with the allergic group, which showed an outstanding increase of it. Hisazumi suggested a relationship between the allergic seminal vesiculitis and the enhanced fibrinolytic activity of the semen which this paper has proved correct. This experiment, however, has something to be completed in human.

As to the efficacy of anti-plasmin drugs for the treatment of allergies, there have been some reports on EACA treatment for anaphylaxis, i. e. penicillin shock etc. When Igai injected trans-AMCHA intravenously to rabbits with experimental allergic arthritis, a slight repression of the enhanced tissue activator activity was observed, but it had no effect on arthritis. When injected into the local site, however, both EACA and trans-AMCHA showed repressive effects. Horiuchi (1968) studying the local fibrinolytic enzyme system by experimental dog arthritis, observed a depression of the fibrinolytic enzyme system by injecting trans-AMCHA, but there was no difference of histological findings between the recipients and non-recipients of trans-AMCHA. Kawai obtained no effective result when trans-AMCHA was injected for experimental nephritis of rabbits. The present experimental study also pointed out the same result. A slight depression of fibrinolytic activity and inflammation can be observed by injecting trans-AMCHA to the local site. With these facts, it is believed that the fibrinolytic enzyme system plays a role in the establishment of allergic inflammation.

Roheae & Silva (1943) observed the activation of the fibrinolytic enzyme system, when anaphylaxis occurred. Furthermore, he suggested that the anaphylaxis was due to histamine, which was released while the activation of plasmin was induced in response to the antigen-antibody reaction. Hayashi (1965) believed that the allergic inflammation resulted from the release of the active peptides, with this release being due to the activation of the proteolytic enzyme of tissue or circulating blood after the antigen-antibody reaction occurred. Kuroyanagi[114,115] (1951) concluded that the

fibrinolytic enzyme system was activated after the allergic reaction occurred. Besides, the permeability of the capillaries also increased, hence bleeding occurred. The permeability of the capillaries was studied by Menkin[116] (1936). He isolated the "leucotaxin" from the exudate at the site of the allergic reaction in rabbits, and also extracted histamine. Therefore, histamine, acetylcholine and bradykinin etc. were proved to exist as the chemical mediators in the tissue of allergic reactions. Kawai made a study concerning experimental nephritis, and ensured that the fibrinolytic enzyme system indirectly caused the establishment of nephritis, which actually was directly caused by the kinin-releasing system; for these were automatically activated by the already activated fibrinolytic enzyme system. Hayashi injected the euglobulin fraction which was obtained from the tissue extraction at the site of allergic reaction, into another place subcutaneously, which showed a reaction resembling that of the Arhtus' phenomenon. Frey et al.[117] (1950) injected bradykinin into the site of the allergic reaction, and observed the inflammation became severe. With this, he confirmed the importance of the kinin-releasing system in the allergic reaction. Kuroyanagi[118] (1969) believed that the above-mentioned chemical mediators were the trigger for activating the fibrinolytic enzyme system, and made this clear to the generally accepted concept. From the fact that the fibrinolytic enzyme system actually acts upon the kinin-forming system, he confirmed that histamine was not released if only by the fibrinolytic enzyme system.

It is easy to understand that the process of allergic inflammation following to the antigen-antibody reaction is very complicated. From the reports of different researchers, the mechanism of allergic inflammation is summarized as follows: after the antigen-antibody reaction occurs, the proteolytic enzyme becomes an activated form; this, in turn, activates the promediator, which, in turn, becomes the mediator; the actions of these mediators finally induce the allergic reactions (Fig. 18). Further-

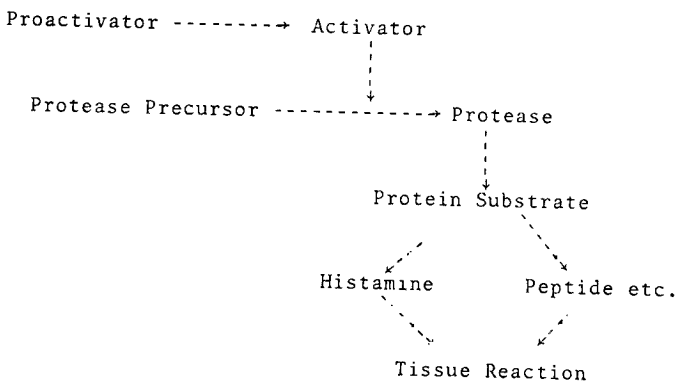


Fig. 18. The mechanism of allergic inflammation (by Kitamura, S., 1959).

more, from the experimental results of mine and other researchers, it is known that anti-plasmin drugs have inhibitory effects only upon the fibrinolytic activity, but not upon the inflammation itself. From these facts, we believe the release reaction of the chemical mediator is not only due to the fibrinolytic enzyme system.

Finally, from the standpoint of antigen-antibody reaction, my present study on the mechanism of the development of hemospermia is concluded as follows : the sensitized seminal vesicles produces the antigen-antibody reaction if in contact with antigen, and this in turn activates the local fibrinolytic enzyme, which causes the release of histamine, bradykinin and other chemical mediators ; consequently, the permeability of the vessels increases and bleeding occurs, which flows into the seminal vesicle lumen and virtually clinical hemospermia ensues. As shown in Fig. 19, the increase of

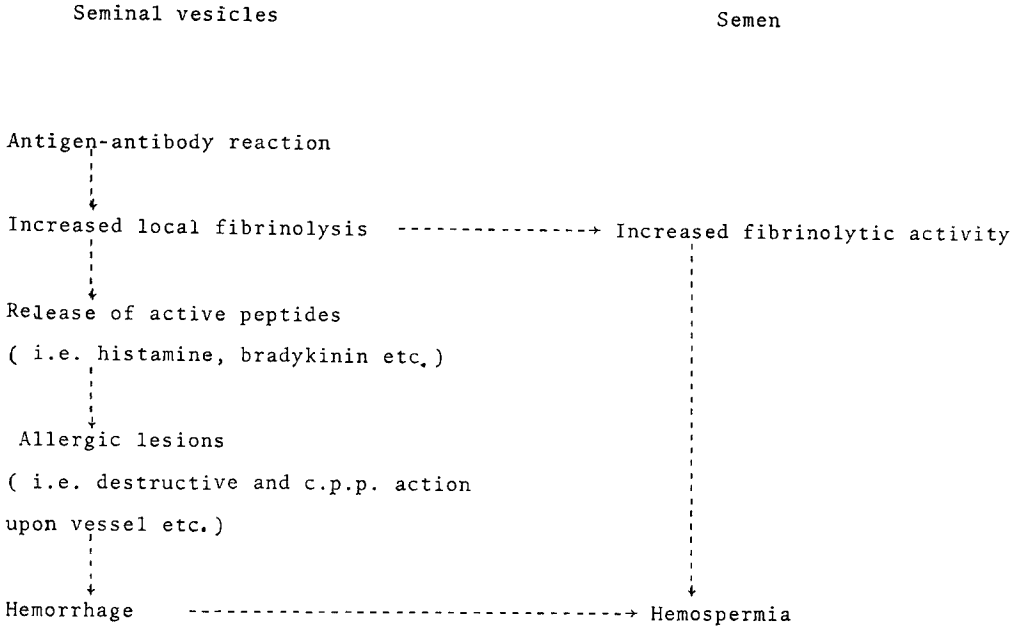


Fig. 19. Simplified scheme of the relationship between allergic and fibrinolytic activity in the seminal vesicles.

fibrinolytic activity at the seminal vesicles is actually revealed as the enhanced fibrinolytic activity of the semen. However, the seminal vesicle allergy is considered as only one of the etiologic factors of essential hemospermia. Furthermore, the seminal vesicle allergy is not believed to be the infectious allergy, but to be autoimmunity which plays a very important part in this disease. Nevertheless, a lot of problems concerning allergic reaction mechanism remains to be elucidated.

Summary

For the purpose of clarifying the pathogenesis of essential hemospermia, the present author made an experimental study of the fibrinolytic activity related to the allergic reactions in the seminal vesicles. For these experiments, allergic reactions in the seminal vesicles of rabbits were induced by means of the complete adjuvant method using egg albumin as an antigen. The estimation of the content of tissue activator was determined by Astrup and Albrechtsen's method and localization in the tissue was topographically observed as described by Todd. In addition, the influence of the allergic reactions upon the fibrinolytic activities of the vesicle fluid and blood were also investigated. The relation of these results to the pathogenesis of essential

hemospermia has been briefly discussed.

The conclusions are as follows:

1. After the injection of the antigen through the vas deferens for the purpose of inducing allergic reactions in the seminal vesicles, microscopic observation of the histological sections revealed submucous edema, petechia, congestion and the infiltration of eosinophils.

2. No significant difference in the plasmin activity of the blood was observed before or after the induction of the allergic reactions in the seminal vesicles. This indicated that the local allergic reactions did not influence the general fibrinolytic enzyme system.

3. Concerning the content of tissue activator, the group with allergic reactions showed a significantly higher content than the control group.

4. In the topographical observation of the seminal vesicles in the group of allergic reaction, the apparent focal lysis areas, specifically indicating the presence of plasminogen activator, corresponded with the submucous layer having histological allergic reactions. Moreover, a remarkable reduction in the focal lysis time was noted in comparison with the control group. However, it was impossible to observe the correlation of histological components, such as the small vessels, and the initial stage of the focal lysis development.

5. The fluid secreted from the seminal vesicles with allergic reactions showed a prominent rise in fibrinolytic activity in comparison with the control group. It is reasonable to presume that this enhanced fibrinolytic activity was due to the release of plasminogen activator from the tissue with allergic reactions.

6. After the induction of allergic reactions in the seminal vesicles, trans-AMCHA (Transamin) was injected via the vas deferens into the seminal vesicles, thereby causing a noticeable reduction of tissue activator in the seminal vesicles and a slight reduction of allergic reactions. The intravenous injection of Transamin, however, resulted in very little inhibitory effects upon the tissue activator and no effect upon the allergic inflammation.

7. According to the above-mentioned results, it is logical to believe that antigen-antibody reaction in the seminal vesicles resulted in local fibrinolysis accompanied by the release and production of some biochemically active agents, and allergic inflammation with hemorrhagic changes.

It is of considerable practical significance that clinically observed essential hemospermia may be caused by allergic lesions in the seminal vesicles.

Acknowledgements

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特発性血精液症に関する実験的研究

—とくに線溶系の立場から—

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特発性血精液症は副性器に器質的変化を示さず，その病因はまだ不明確であるが，近年精嚢アレルギーの関与が重要視されている．いっぽう泌尿器科領域において，とくに腎，前立腺，精液などにおける局所線溶能の研究は臨床病理上注目されている．

著者は動物実験において精嚢アレルギーを作成し，その組織学的観察とともに，線溶系の立場から，精嚢組織，分泌液などを検討し2，3の知見を得たので報告する．

実験動物として2kg以上の雄性成熟家兎を用い，アジュバント法により結晶卵白アルブミン（5 mg/kg）を用いて，実験的精嚢アレルギーを作成した．肉眼的に精嚢粘膜面は高度の浮腫とともに小出血斑を示し，顕微鏡的には粘膜下浮腫，溢血，充血，毛細血管拡張，好酸球浸潤などのアレルギー性反応を認めた．Astrupの方法に準じたロダンカリ抽出法により，精嚢組織のplasminogen activator contentを測定し，対照群に比してアレルギー群に有意の高値を認めた．さらにこれら組織におけるplasminogen activatorの局在をTodd法に準じて，組織化学的に観察した．すなわちアレルギー性変化の著しい粘膜下層に一致してfibrin filmのfocal lysisが見られ，対照群に比してfocal lysisの出現時間に明らかな短縮が認められた．いっぽう精嚢摘出のさい，同時に採取した精嚢分泌液についても，対照群に比し，アレルギー群に明らかな線溶能亢進を認めた．さらにtrans-aminomethylcyclohexane carboxylic acidの局所投与により，これら亢進せる線溶能は全身投与例に比し強く抑制されるが，アレルギー性炎症はごく軽度抑制されるに過ぎなかった．アレルギー反応惹起前後の血中線溶能に有意の変動が認められず，アレルギー反応局在部における線溶系の増強が著明に認められた．

以上の実験成績は臨床例において精液線溶能亢進を認め（教室・久住），かつ抗プラスミン剤の効果が著しいことと一致し，特発性血精液症発症の一機序として精嚢アレルギー，線溶系の関与が示唆されるもので，この問題について考察をおこなった．

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